



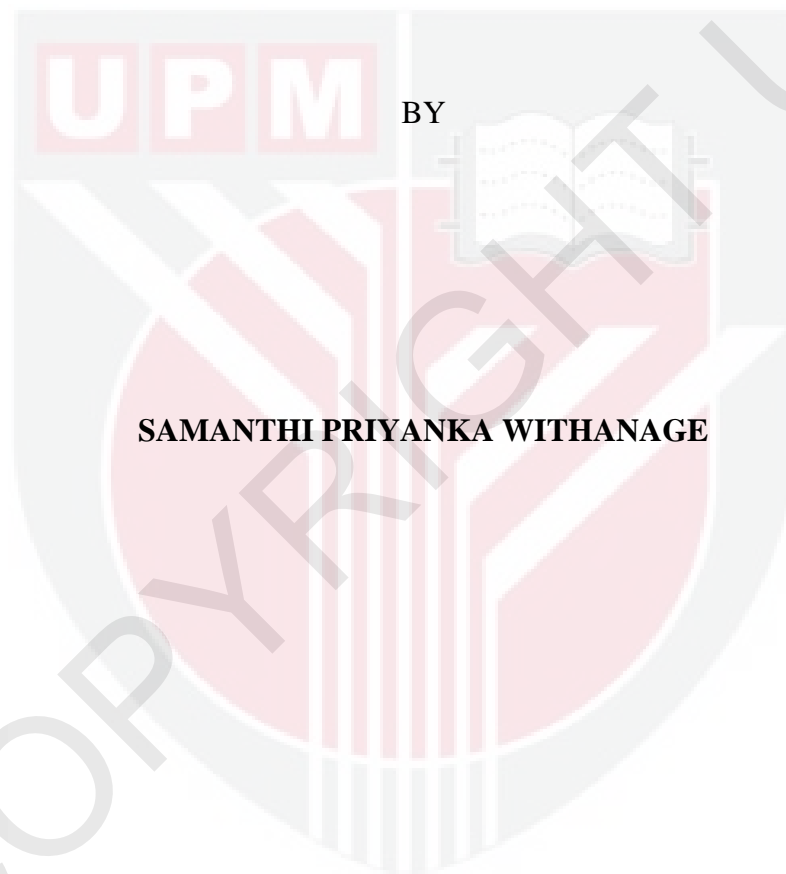
UNIVERSITI PUTRA MALAYSIA

***OVER-EXPRESSION OF GIBBERELLIN-20 OXIDASE GENE IN KENAF
(Hibiscus cannabinus L) FOR INCREASED FIBER QUALITY***

SAMANTHI PRIYANKA WITHANAGE

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SAMANTHI PRIYANKA WITHANAGE

Thesis Submitted to the School of Graduate Studies, Universiti Putra
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Doctor of Philosophy

May 2012

DEDICATION

My late mother Daya Rajapaksa,

Father Srimapala Withanage

Husband Kalyanapriya Ramanayake

and

My lovely daughters

Pamuditha and Ravishika

Abstract of thesis presented to the senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

**OVER-EXPRESSION OF GIBBERELLIN- 20 OXIDASE GENE IN KENAF
(*Hibiscus cannabinus* L) FOR INCREASED FIBER QUALITY**

By

SAMANTHI PRIYANKA WITHANAGE

May 2012

Chairman : Assoc. Prof. Suhaimi B Napis, PhD

Faculty : Biotechnology and Biomolecular Sciences

Kenaf (*Hibiscus cannabinus* L.) is a multipurpose herbaceous crop belongs to the family Malvaceae. It is one of the potential alternatives of natural fibers for biocomposite production including pulp and paper. Kenaf generally grows very fast in the tropics. The kenaf stem consists of long bast (represents 34 - 38% of the stem) and short core fibers (represents 62 - 66% of the stem). The success of the kenaf fiber used industries has relied upon its high yield per hectare and, the quality and quantity

of its bast and core fibers. Current data on the yield of kenaf fibers shows that there is still plenty of room for improvement. Therefore, the development of improved kenaf planting materials is one of the areas where more research should be focused upon. Ideally, longer fiber length indicated by short core fiber and higher cellulose content are required for high quality kenaf fiber. In plants, gibberellic acid (GA) which is an important plant hormone influences the structural development of a plant and its organs. The hormone stimulates cell division and elongation, and promotes transition of vegetative to reproductive growth. Therefore, in this study, it is hypothesized that by increasing the active GA, the fiber length and cellulose content (biomass) of kenaf would be increased. The hypothesis was tested by evaluating the effects of over expression of gibberellin 20 oxidase (GA20ox) gene, one of the key enzymes in GA biosynthetic pathway in kenaf.

Two forms of GA 20 oxidase gene i.e. a gene with intron (AtGA20ox-In) and a gene without the intron (AtGA20ox-cDNA) were isolated from *Arabidopsis thaliana* ecotype Columbia and overexpressed in kenaf under the control of the double CaMV 35S promoter. This was followed by *in planta* (*in vitro*) transformation into the V36 (intermediate flowering) and G4 (late flowering) varieties of kenaf. The putative transformants over expressing AtGA20ox gene were screened for hygromycin B resistance and confirmed by PCR and Southern blot analysis. The transgene transcripts of the transgenic kenaf were analyzed by real time PCR, and the levels of bioactive GA₁ and GA₄ were determined by GC-MS analysis. The lines that showed

higher levels of bioactive GA (0.3-1.52 ng/g fresh weight) were chosen for further characterization of their morphological and biochemical traits including vegetative and reproductive growth, fiber dimensions and chemical composition.

Different levels of GA20ox expression were observed in the transgenic lines ranging from 2 to 39 fold increases of the transcripts level. The expression level was correlated positively with the production of bioactive GA₁ and GA₄. Various types of phenotypes as short non flowering, short early flowering, normal flowering and tall non flowering were observed among the transgenic lines in both of the varieties transformed. Two transgenic lines (V36-2 and V36-3) out of four lines produced high levels of GAs (1.43 and 1.23 ng/g fresh weight) flowered at 7th week. It was considered as very early compare to untransformed plants which flowered at 13th week. However, these lines were unable to complete their reproductive phase resulting in poor seed production. The other two lines (G4-5 and G4-7) showed impaired vegetative and reproductive growth characterizing by lack of reproductive phase. It is speculated that the lines might be subjected to gibberellins homeostasis where those with normal levels of GAs (around 0.3ng/g fresh weight) grew phenotypically normal, whereas the lines that produced slightly higher concentrations of GAs (0.32-0.52 ng/g fresh weight) produced better vegetative growth which exhibited delayed in flowering or failed in initiating flowering. Positive impact of gibberellins on biochemical composition, fiber dimensions and their derivative values of kenaf was demonstrated in some lines of the transgenic

kenaf including the increased cellulose content of 91% and increased fiber length of 3.22 mm for bast fiber and 1.1mm for core fiber. It has been noticed that the levels of bioactive GA₁ and GA₄ have influence in determining the vegetative growth and reproductive development of kenaf. But it required a further detailed study to confirm the critical level of this bioactive GA. This study confirmed the hypothesis that GA is extremely important for increasing the length of the kenaf fiber. The findings can be used as a basis for further improving the quality and quantity of kenaf in the industry.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**PENG-EKSPRESAN LAMPAU GEN GIBBERELLIN- 20 OKSIDASE
DALAM KENAF (*Hibiscus cannabinus* L) UNTUK PENINGKATAN KUALITI
SERAT**

Oleh

SAMANTHI PRIYANKA WITHANAGE

Mei 2012

ABSTRAK

Pengerusi : Prof Madya Suhaimi B Napis, PhD

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Kenaf (*Hibiscus cannabinus* L.) adalah tanaman pelbagaiguna yang digolongkan di bawah famili Malvaceae. Serat atau gentian aslinya merupakan salah satu alternatif yang berpotensi untuk penghasilan biokomposit termasuk pulpa dan kertas. Kenaf umumnya cepat tumbes di kawasan tropika. Batang kenaf terdiri daripada bahagian 'bast' yang mempunyai serat yang panjang (mewakili 34 - 38% daripada batang) dan bahagian teras yang mempunyai serat yang pendek (mewakili 62 - 66% daripada batang). Kejayaan industri berasaskan serat kenaf bergantung kepada penghasilan kenaf yang tinggi per hektar dan juga kualiti dan kuantiti serat 'bast' dan terasnya. Merujuk kepada data semasa penghasilan serat kenaf jelas menunjukkan

bahawa masih terdapat banyak ruang untuk penambahbaikan ke atas tanaman ini. Oleh itu, pembangunan stoktanaman kenaf yang lebih baik merupakan salah satu bidang penyelidikan yang sepatutnya diberikan tumpuan. Sebaiknya penambahan panjang serat teras yang selalunya pendek dan peningkatan kandungan selulosa yang lebih tinggi merupakan keperluan penting bagi serat kenaf yang berkualiti tinggi. Hormon 'Gibberellic acid' (GA), merupakan hormon tumbuhan yang penting dalam mempengaruhi pembangunan struktur tumbuhan dan organnya. Hormon ini merangsangkan pembahagian dan pemanjangan sel, dan menggalakkan peralihan fasa pertumbuhan vegetatif kepada fasa pembiakan. Oleh itu, hipotesis dalam kajian ini adalah apabila kandungan 'GA' aktif meningkat, maka panjang serat dan kandungan selulosa (biojisim) dalam kenaf juga akan meningkat. Hipotesis ini telah diuji dengan menilai kesan daripada pengepresan lampau gen gibberellin 20 oksidase (GA20ox), yang merupakan salah satu daripada enzim utama dalam laluan biosintetik GA dalam kenaf. Dua bentuk gen GA 20 oksidase iaitu gen bersama intron (AtGA20ox-In) dan gen tanpa intron (AtGA20ox-cDNA) telah diasingkan daripada *Arabidopsis thaliana* jenis Colombia dan pengepresan lampau dalam kenaf adalah di bawah kawalan promoter berganda CaMV 35S. Ini diikuti dengan transformasi secara '*in planta*' ('*in vitro*') ke atas dua varieti kenaf iaitu V36 (berbunga pertengahan) dan G4 (berbunga lewat). Transformasi yang disasarkan ke atas gen AtGA20ox disaring menggunakan antibiotik hygromycin B dan disahkan melalui analisis PCR dan penghibridan 'Southern'. Transkrip transgen berkenaan dalam kenaf transgenik dianalisis dengan PCR masa-nyata, dan tahap kandungan bioaktif GA₁ dan GA₄ ditentukan melalui analisis GC-MS. Kenaf transgenik yang menunjukkan tahap GA yang tinggi (0.3-

1.52ng/g berat bersih) telah dipilih untuk pencirian lanjut dari segi morfologi dan biokimia termasuklah fasapertumbuhan vegetatif dan pembiakan, dimensi dan komposisi erat masing.

Tahap ekspresi GA20ox yang berbeza telah diperhatikan dalam kenaf transgenik iaitu kenaikan di antara 2-39 kali ganda berbanding kawalan. Tahap ekspresi tersebut adalah berkorelasi secara positif dengan pengeluaran bioaktif GA₁ dan GA₄. Pelbagai jenis fenotip diperhatikan di kalangan progeni kenaf transgenik di dalam kedua-dua varieti yang telah ditransformasikan. Dua (V36-2 dan V36-3) daripada empat jenis kenaf transgenik yang menghasilkan tahap GA yang lebih tinggi (1.43 dan 1.23 ng/ g berat bersih), berbunga pada minggu ketujuh. Ia dianggap sebagai sangat awal berbanding dengan tumbuhan yang tidak ditransformasikan yang hanya mula berbunga pada minggu ke-13 dan mereka tidak dapat melengkapkan fasa pembiakan yang menyebabkan pengeluaran benih yang lemah. Dua transgenik yang lain pula (G4-5 dan G4-7) menunjukkan pertumbuhan vegetatif dan pembiakan yang terjejas. Kejadian ini berlaku, mungkin disebabkan oleh 'homeostasis gibberellin' di mana transgenik yang mempunyai tahap GA yang biasa (0.3ng/g berat bersih) menjana fenotip normal, sedangkan bagi mereka yang menghasilkan GAs yang tinggi (0.32-0.52 ng/g berat bersih) mempunyai pertumbuhan vegetatif yang lebih baik iaitu yang menunjukkan lambat berbunga atau tidak berbunga langsung. Kesan positif GA pada kandungan biokimia dan dimensi serat, dan nilai-nilai terbitan kenaf telah juga ditunjukkan dalam beberapa kenaf transgenik tersebut seperti peningkatan kandungan selulosa setinggi 91% dan pemanjangan serat sebanyak 3.22 mm untuk

serat ‘bast’ dan 1.1 mm untuk serat teras . Adalah didapati bahawa tahap GA adalah sangat kritikal dalam menentukan fenotip kenaf dan ini memerlukan kajian lanjutan yang lebih terperinci. Kajian ini telah mengesahkan hipotesis bahawa GA adalah sangat penting untuk meningkatkan panjang serat kenaf. Hasil kajian ini boleh digunakan sebagai asas untuk terus meningkatkan kualiti dan kuantiti kenaf di negara ini yang secara tidak langsung boleh menyumbang kepada perkembangan industri berasaskan serat kenaf.



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I certify that a Thesis Examination Committee has met on 24th May 2012 to conduct the final examination of Samantha Priyanka Withanage on her thesis entitled “**Over-expression of Gibberellin- 20 oxidase gene in kenaf (*Hibiscus cannabinus* L.) for increased fiber quality**” in accordance with the Universities and University Collages Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The committee recommends that the student awarded the Doctor of Philosophy.

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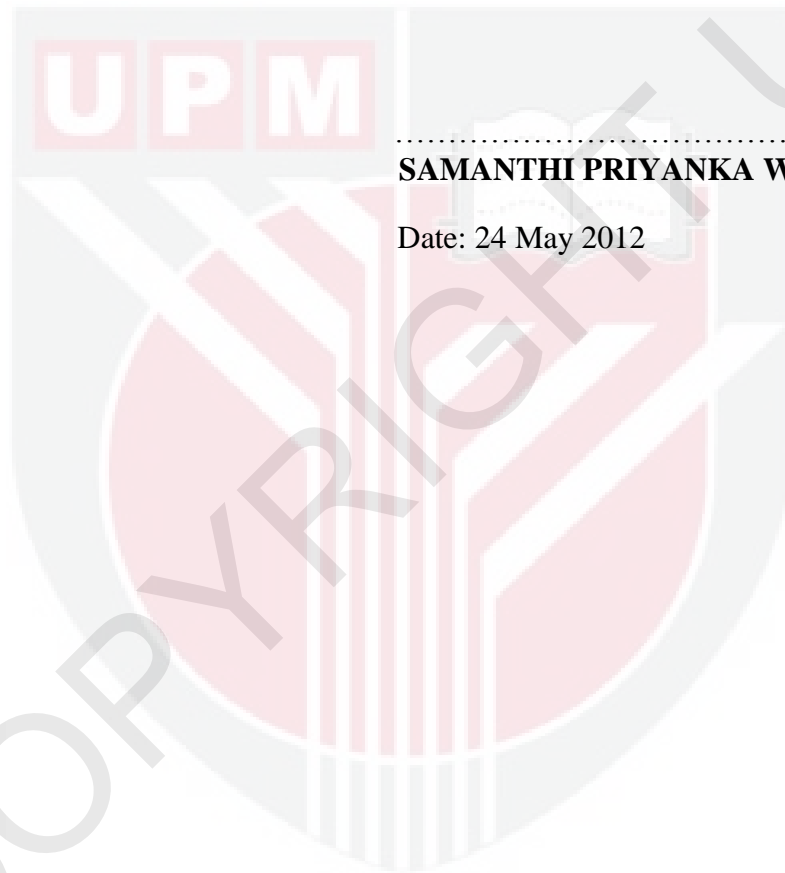
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DECLARATION

I declare that the thesis is my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.



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SAMANTHI PRIYANKA WITHANAGE

Date: 24 May 2012

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